Agrobacterium Mediated Genetic Transformation in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.) for Stress Tolerance

A Synopsis

Submitted in

partial fulfillment for the degree

of

Doctor of Philosophy

(Biotechnology)

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March, 2012

Introduction:

Pennisetum glaucum (L.) R. Br. is commonly known as Pearl millet, grown mostly in the tropics and subtropics of the world. Taxonomically Pearl millet belongs to the family Poaceae, subfamily Panicoideae and genus Pennisetum. It is the fourth most important cereal crop to be cultivated after rice, wheat and sorghum. In Rajasthan it is the principal grain crop grown on 53.8% of the cereal area and 67% of the coarse grain area. It is a rainfed crop sown with the first rain during the growing season.

There are basically main constraints in increasing the productivity of pearl millet are classified into two first is abiotic stress and second is biotic stress. Both abiotic and biotic stress factors have been estimated to cause an estimated loss of US $ 15.75 billion. Environmental factors such as high temperature, light intensity, salinity, drought and availability of nutrients play an important role in plants growth and development. Any variation in these above stated factors which leads to decline in the growth and productivity of plants is termed as abiotic stress (Levitt 1980). Biotic stress includes the bacterial, fungal or pests attack on plants which reduces growth and productivity. Examples of fungal disease- Downy mildew disease caused by *Sclerospora graminicola* (Sacc) Schort, Ergot caused due to *Claviceps fusiformis* and seed borne fungal disease are the most serious constraint in grain yield in Pearl millet (Neya and Kabore 1987). Downy mildew of Pearl millet is a typical case of inflorescence malformation and conversion of florets into green leafy structures. Low soil fertility (high salt concentration), inadequate rainfall (drought) and prevalence of diseases (fungal) are the major causes of low survival and productivity.

Salinity affects more than 10% arable land on global scale (Bray et al. 2002). Year after year more land under irrigation is becoming increasingly saline. About 20% of worlds cultivated land and nearly half of the worlds irrigated area are inflicted to salinity (FAO 2005). Drought is also most devastating production constraint that can occur at any growth stage in Pearl millet. An estimated loss of 10-60% of Pearl millet harvest has been reported due to fungal infection.

Both abiotic and biotic stresses affects the morphological, physiological and biochemical processes. Morphological symptoms include reduced seed germination, reduced seedling growth, and reduced dry matter content (Munns et al. 1982, Hasegawa 2000). Under abiotic and biotic stress a series of physiological an biochemical changes occur which includes increased production of Osmoprotectant (proline, free amino acid and glycine betain), increased activity of antioxidant enzymes (catalase, Ascorbate peroxidase, Superoxide dismutase, Peroxidase, Polyphenol oxidase and many others) (Alscher 2002;Hasegawa 2000).

Rice chitinase gene have been transferred in many plants to using suitable vector to confer tolerance against fungal infection, plants resists pathogen attack by accumulating chitinases protein which is active against the disease causing organism. In some cases, where this mechanism is too weak or appears too late to fully protect the plant, engineering constitutive expression of a defenses protein can boost tolerance to fungal pathogens (Broglie et al. 1991, Grison et al. 1996). Develop transgenic Pearl millet with resistance to fungal disease will help in increasing the productivity.

Importance of Study

Environmental stress is a major problem across the world. In several cases, scientists have used genetic engineering to develop plants with enhanced tolerance against environmental stressed conditions. This work will help in solving the above stated problem. The transgenic Pearl millet obtained after genetic transformation will have enhanced level of proteins/enzymes which will improve the tolerance against stressed condition and thus the plants will be capable of growing in the stressed condition. Biolistic–mediated gene delivery has been frequently used for the transformation of millets but *Agrobacterium tumefaciens*-mediated transformation is still lagging. Improved transformation of millets, allied to relevant gene targets which may offer resistance to environmental stress and will play important role in millet improvement. Development of transgenic plants using biotechnological tools has become important in plant-stress biology. The various abiotic stresses cause changes in plant processes at all levels of organization i.e. morphological, physiological, biochemical and molecular. The understanding of mechanisms that regulate gene expression and the ability to transfer genes from other organisms into plants will expand the ways in

which plants can be utilized. The use of novel approaches combining genetic, physiological, biochemical and molecular techniques should provide excellent results in the near future. The task of generating transgenic cultivars is not only limited to the success in the transformation process, but also proper incorporation of the stress tolerance. Evaluation of the transgenic plants under stress conditions, and understanding the physiological effect of the inserted genes at the whole plant level remains as a challenge.

Objectives:

1. To study the effects of abiotic and biotic stress on Pearl millet.

2. To generate a protocol for *in-vitro* regeneration using suitable explant.

3. Agrobacterium mediated gene transfer of rice chitinase gene.

4. Subsequent selection and regeneration of transformed explants using a positive selectable marker.

5. Molecular analysis of putative transformants.

6. Evaluate the effect of abiotic and biotic stress on the transformed Pearl Millet *in vitro.*

Review of Literature:

Many scientists across the world have contributed in the field of *in-vitro* culture of Pearl millet through organogenesis and somatic embryogenesis from different explants (immature and mature embryos, leaf base, inflorescence and shoot apices)(Devi et al., 2000; Devi et al. 2001, Mythili et al., 2001; Oldach et al., 2001; Srivastava and Kothari, 2002 and Arockiasamy et al., 2006).

The study of effect of environmental stress (abiotic and biotic stress) on Pearl Millet few of them have been mentioned in this review. Under drought conditions a decrease in leaf water potential and osmotic potential have been reported. Free proline and total free amino acid accumulation have also been reported under drought, salinity and fungal infection. An increased activity of antioxidant enzymes (catalase, Ascorbate peroxidase, Superoxide dismutase, Acid and alkaline phosphatase, poly phenol oxidase and peroxidase have also been reported (Ashraf et al 2001; Johnsi Rani 2011; Jain et al. 2004; Arun et al. 2010).

Various genes have been reported for increasing the tolerance against the biotic stress in many plants. A list of few genes conferring fungal tolerance has been given in table below.

Sr. no.	**plant**	**Pathogen Related protein**	**Fungus tested**	**references**
1	Alfalfa	PR2 (class II glucanase)	*Phytopthora megasperma*	Masoud et al. 1996
2	Canola	PR3 (class I chitinase)	*Rhizoctonia solani Pythium aphanidermatum*	Broglie 1991
3	Grapevine	PR3 (class I chitinase)	*Elisinoe ampelina*	Yamamoto et al. 2000
4	Tobacco	PR3 (class II chitinase)	R. *solani*	Jach et al. 2005
5	Wheat	PR3 (class II chitinase)	*Erysiphe graminis*	Bliffeld et al 1999
6	Carrot	PR3 (class I chitinase) and PR2 (class I glucanase)	*Alternaria dauci Alternaria radicina Cercospora carotae Erisyphe heraclei*	Stuiver et al. 1996

Broglie et al. (1991) showed that both transgenic tobacco and Brassica napus exhibited enhanced resistance towards Rhizoctonia solani, following constitutive expression of the chitinase gene. Transgenic peanut plants following transformation

with tobacco chitinase, showed partial resistance to *Cercospora arachidicola* (Rohini and Rao 2001). When Salehi et al. (2005) introduced bean chitinase gene in soybean driven by a constitutive promoter, they obtained a few transgenic lines that showed greater chitinase activity compared to un-transformed plants towards *Rhizoctonia solani* during an in vitro assay. Epple et al. (1997) transformed Arabidopsis thaliana with Arabidopsis endothionin Thi2.1 gene and found enhanced resistance in the transgenic against *Fusarium oxysporum* f. sp. *matthiolae*, whereas, the untransformed Arabidopsis showed no defense against the same fungus.

The rice chitinase gene harboured in the plasmid pCAMBIA 1302:RChit have been transferred in peanut, pigeonpea, fingermillet, grape wine, cotton and sorghum to confer fungal tolerance (Prasad et al. 2012; Nirala et al 2010; Ignacimuthu and Ceasar, 2012; Kumar et al. 2004; Iqbal 2012; Ganesan et al.2009).

Methodology:

1. **To study the effects of abiotic and biotic stress on Pearl millet:** treating the Pearl millet seedlings with different concentrations of NaCl, Polyethylene glycol-6000 and mycoflora for short duration to study the morphological parameters (germination efficiency, growth parameters), biochemical parameters (proline and free amino acid estimation), Antioxidative enzyme activities (Catalase, Ascorbate peroxidase and Superoxide dismutase) and protein profiles (protein extraction, protein estimation and SDS-PAGE).

2. **To generate a protocol for *in-vitro* regeneration using suitable explant:** culturing the explant of Pearl millet on MS media with different growth regulators.

3. **Agrobacterium mediated gene transfer of rice chitinase gene harboured in the plasmid pCAMBIA 1302:RChit in explant.**

4. **Subsequent selection and regeneration of transformed explants using a positive selectable marker:** hygromycin selection.

5. **Molecular analysis of putative transformants.**

6. **Evaluate the effect of abiotic and biotic stress on the transformed Pearl millet:** treating the transformed Pearl millet seedlings expressing putative gene with different concentrations of NaCl, Polyethylene glycol-6000 and mycoflora for short duration to study the morphological parameters (germination efficiency, growth parameters), biochemical parameters (proline and free amino acid estimation), Antioxidative enzyme activities (Catalase, Ascorbate peroxidase and Superoxide dismutase) and protein profiles (protein extraction, protein estimation and SDS-PAGE).

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